ENT COOPERATION TRE

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BERESKIN & PARR
40th floor
40 King Street West
Toronto, Ontario M5H 3Y2
CANADA

Date of mailing (day/month/year)
13 July 2001 (13.07.01)

Applicant's or agent's file reference
6857-7

IMPORTANT NOTIFICATION

International application No.
PCT/CA99/01157

International filing date (day/month/year)
03 December 1999 (03.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
BIONICHE INC.
383 Sovereign Road
London, Ontario N6M 1A3
Canada

State of Nationality: CA
State of Residence: CA
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
BIONICHE LIFE SCIENCES INC.
383 Sovereign Road
London, Ontario N6M 1A3
Canada

State of Nationality: CA
State of Residence: CA
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740 14.35

Authorized officer
F. Baechler
Telephone No.: (41-22) 338.83.38

P ~ 'ENT COOPERATION TREAT~

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 August 2000 (21.08.00)	
International application No. PCT/CA99/01157	**Applicant's or agent's file reference** 6857-7
International filing date (day/month/year) 03 December 1999 (03.12.99)	**Priority date** (day/month/year) 04 December 1998 (04.12.98)
Applicant PHILLIPS, Nigel, C. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 June 2000 (21.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT IB 331 (July 1992)

CA9901157

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 45/06, A61P 31/00 — A1

(11) International Publication Number: WO 00/33875

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/CA99/01157

(22) International Filing Date: 3 December 1999 (03.12.99)

(30) Priority Data:

60/111,019	4 December 1998 (04.12.98)	US
60/127,320	1 April 1999 (01.04.99)	US

(71) Applicant *(for all designated States except US)*: BIONICHE INC. [CA/CA]; 383 Sovereign Road, London, Ontario N6M 1A3 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: PHILLIPS, Nigel, C. [CA/CA]; 101 Seigniory Avenue, Point–Claire, Quebec H9R 1J6 (CA). FILION, Mario, C. [CA/CA]; 2377 St. Zotique, Montreal, Quebec H2G 1K3 (CA).

(74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHEMOTHERAPEUTIC COMPOSITION AND METHOD

(57) Abstract

The present invention relates to a composition and method comprising *Mycobacterium phlei (M. phlei)*–DNA (M–DNA), M–DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the M–DNA and the MCC induce cell cycle arrest in proliferating cancer cells, inhibit proliferation of cancer cells, induce apoptosis in cancer cells and potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01157

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S.YAMAMOTO E.A.: "In vitro augmentation of natural killer cell activity and production of interferon alpha/beta and -gamma with deoxyribonucleic acid fraction from mycobacterium bovis BcG" JAPANESE JOURNAL OF CANCER RESEARCH, vol. 79, 1988, pages 866-873, XP002085535 page 866 page 872, column 1 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 2000	18/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Peeters, J

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US FILION, M. C. (1) ET AL: "Mycobacterial cell wall-DNA complex induces apoptosis in cancer cells." retrieved from STN XP002133100 abstract & JOURNAL OF PHARMACY AND PHARMACOLOGY, (SEPT., 1998) VOL. 50, NO. SUPPL., PP. 39. MEETING INFO.: 135TH MEETING OF THE BRITISH PHARMACEUTICAL CONFERENCE EASTBOURNE, ENGLAND, UK SEPTEMBER 8-11, 1998 , ---	1,2
P,X	WO 99 07383 A (BIONICHE) 18 February 1999 (1999-02-18) claims 1-3,5,6,9-11 page 10, line 29-31 page 11, line 23-28 -----	1-42

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CA 99/01157

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9907383 A	18-02-1999	AU 8723698 A	01-03-1999
		AU 1746599 A	06-09-1999
		WO 9942113 A	26-08-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
6857-007		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/01157	03/12/1999	04/12/1998
International Patent Classification (IPC) or national classification and IPC A61K45/06		
Applicant BIONICHE LIFE SCIENCES INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

   4

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/06/2000	22.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cattell, James Telephone No. +49 89 2399 8468

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/01157

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-14,17,18	as originally filed			
15,16	as received on	15/12/2000	with letter of	15/12/2000

Claims, No.:

1-18	as received on	15/12/2000	with letter of	15/12/2000

Drawings, sheets:

1/9-9/9	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 2,3,6,9-16,18.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 2, 9,10, 12, 13, 15, 16, 18 (see separate sheet). are so unclear that no meaningful opinion could be formed (*specify*): **see separate sheet**

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3, 6, 10, 12, 13, 16. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;

citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,4,5,7,8,17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1,4,5,7,8,17
Industrial applicability (IA)	Yes:	Claims	1,4,5,7,8,17
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

III.

1). There appears to be 10 from 18 independent claims. This is totally unreasonable under Rule 6 PCT. Furthermore there appears to be no basis in the original application under Article 34 PCT given for new claims 3, 6 (in cancer cells in general),12, 13, for the use of MCC without M-DNA (claims 10, 16 and 18).
As these claims have not been searched and the scope of protection sough is fully unclear to the IPEA, the Examination can only therefore be based on the first independent claim in each Category, i.e. claims 1 and 17 (Article 34(4)(a) and Rule 66(1)(e) PCT).

V.

2). Document D1 (Filion et al 1998 J.Pharm.Pharmacol. 50;39, enclosed) discloses that DNA from mycobacterium phlei (M-DNA) has been shown to induce apoptosis in cancer cells.
The use of M-DNA with another chemotherapeutic agent is not disclosed, in the prior art. (Art 33(2) PCT).
However it would seem obvious to the skilled man in cancer therapy to combine two known antineoplastic agents. The subject-matter of claims 1, 4, 5, 7, 8 and 17 therefore appear not to meet the requirements of Article 33(3) PCT.

VIII.

3). The amendments to pages 15 and 16 do not meet the requirements of Article 34 PCT. Although there is a contradiction between the amended areas and page 16 paragraph 1, it is not immediately evident which one of the given units is in error.

4). For the assessment of the present claims 1 and 3-8 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

UK

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
6857-7		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 99/ 01157	03/12/1999	04/12/1998
Applicant		
BIONICHE INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/01157

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S.YAMAMOTO E.A.: "In vitro augmentation of natural killer cell activity and production of interferon alpha/beta and -gamma with deoxyribonucleic acid fraction from mycobacterium bovis BcG" JAPANESE JOURNAL OF CANCER RESEARCH, vol. 79, 1988, pages 866-873, XP002085535 page 866 page 872, column 1 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 2000	18/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Peeters, J

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US FILION, M. C. (1) ET AL: "Mycobacterial cell wall-DNA complex induces apoptosis in cancer cells." retrieved from STN XP002133100 abstract & JOURNAL OF PHARMACY AND PHARMACOLOGY, (SEPT., 1998) VOL. 50, NO. SUPPL., PP. 39. MEETING INFO.: 135TH MEETING OF THE BRITISH PHARMACEUTICAL CONFERENCE EASTBOURNE, ENGLAND, UK SEPTEMBER 8-11, 1998 , ---	1,2
P,X	WO 99 07383 A (BIONICHE) 18 February 1999 (1999-02-18) claims 1-3,5,6,9-11 page 10, line 29-31 page 11, line 23-28 -----	1-42

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 99/01157

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9907383 A	18-02-1999	AU 8723698 A	01-03-1999
		AU 1746599 A	06-09-1999
		WO 9942113 A	26-08-1999

Mitomycin-C is an anti-tumor antibiotic produced by Streptomyces caespitosus, which cross-links DNA, depolymerizes DNA and forms free radicals.

Figure 2 shows that, with B-16 cells, 0.1 μg/ml mitomycin-C inhibited proliferation about 5%, 1 μg/ml about 10%, and 10 and 100 μg/ml 100%, whereas 1 μg/ml MCC inhibited proliferation about 25%, 10 μg/ml about 50% and 100 μg/ml about 80%. Figure 2 also shows that, in the presence of 1 μg/ml MCC, 0.1 μg/ml mitomycin-C inhibited proliferation about 40%, 1 μg/ml about 65% and 100 μg/ml 100%. These data show that MCC potentiates the antineoplastic effect of mitomycin-C on proliferating cancer cells.

B-16 melanoma cells were incubated with 0.01 to 100 μg/ml of 5-fluorouracil, with 1 to 100 μg/ml of MCC and with 0.01 to 10 μg/ml of 5-fluorouracil + 1 μg/ml MCC. 5-fluorouracil is an antimetabolite, which interferes with DNA and RNA synthesis.

Figure 3 shows that, with B-16 cells, 0.01μg/ml 5-fluorouracil inhibited proliferation about 8%, 0.1 μg/ml about 50%, 1 μg/ml about 90%, and 10 and 100 μg/ml 100%, whereas 1 μg/ml MCC inhibited proliferation about 25%, 10 μg/ml about 50% and 100 μg/ml about 80%. Figure 3 also shows that, in the presence of 1 μg/ml MCC, 0.01 μg/ml 5-fluorouracil inhibited proliferation about 75%, 0.1 μg/ml about 85%, 1 μg/ml about 90% and 10 μg/ml 100%. These data show MCC potentiates the antineoplastic effect of 5-fluorouracil on proliferating cancer cells.

B-16 melanoma cells were incubated with 0.01 to 100 μg/ml of cisplatin, with 1 to 100 μg/ml of MCC and with 0.01 to 10 μg/ml of cisplatin + 1 μg/ml MCC. Cisplatin is an alkylating agent that cross-links DNA and inhibits DNA precursors.

Figure 4 shows that, with B-16 cells, 0.01 μg/ml cisplatin inhibited proliferation 0%, 0.1 μg/ml about 8%, 1 μg/ml about 62%, 10 μg/ml about 90% and 100 μg/ml 100%, whereas 1 μg/ml MCC inhibited proliferation about 25%, 10 μg/ml about 50% and 100 μg/ml about 80%. Figure 4 also shows that, in the presence of 1 μg/ml MCC, 0.01 μg/ml cisplatin inhibited proliferation about 40%, 0.1 μg/ml about 50%, 1 μg/ml about 70% and 10 μg/ml about 90%. These data show that MCC enhances the antineoplastic effect of cisplatin on proliferating cancer cells.

Table 5 shows the concentrations of mitomycin-C, 5-fluorouracil, and cisplatin required for 50% inhibition of B-16 melanoma cell division in the absence and in the presence of 1 μg/ml MCC.

Table 5

Concentration of mitomycin-C, 5-fluorouracil and cisplatin required for 50% inhibition of B-16 melanoma cell proliferation in the absence and in the presence of 1 mg/ml MCC

Treatment	IC_{50}*, µg/ml	
	Drug Alone	Drug + MCC at 1 mg/ml
MCC	10	Not applicable
Cisplatin	0.6	0.16
5-Fluorouracil	0.12	0.005
Mitomycin-C	2.2	0.12

*concentration for 50% inhibition

Table 5 shows the dose dependent inhibition of B-16 cell melanoma proliferation by MCC at 10 to 100 µg/ml (IC_{50}=10 µg/ml) and by mitomycin-C, 5-fluorouracil and cisplatin at 0.1 to 10 µg/ml (IC_{50}=2.2, 0.12 and 0.6 µg/ml respectively). Table 5 also shows that 1 µg/ml MCC potentiated mitomycin-C (IC_{50}=0.12 µg/ml) and 5-fluorouracil (IC_{50}=0.005 µg/ml) inhibition of B-16 melanoma cell proliferation and that 1 µg/ml MCC enhanced cisplatin (IC_{50}=0.16 µg g/ml) inhibition of B-16 melanoma proliferation

These data show that MCC not only inhibits cancer cell proliferation, but also potentiates the antineoplastic effects of mitomycin-C and 5-fluorouracil on cancer cell proliferation and enhances the antineoplastic effect of cisplatin on cancer cell proliferation.

EXAMPLE 12

Induction of apoptosis in B-16 melanoma cells by MCC and M-DNA

Fragmentation of cellular DNA into nucleosome-sized fragments is characteristic of cells undergoing apoptosis (Newell et al. Nature 357:286-289, 1990). To assess DNA fragmentation, B-16 cells were lysed with 0.5 ml of hypotonic lysing buffer (10 mM Tris buffer, 1 mM EDTA, 0.2% t-octylphenoxypolyethoxyethanol (Triton X-100), pH 7.5). The lysates were centrifuged at 13,000 g for 10 min and the supernatants, containing fragmented DNA, were precipitated overnight at -20°C in 50% isopropanol and 0.5 M NaCl. The precipitates were collected by centrifugation and were analyzed by electrophoresis in 0.7% agarose gels for 3 h at 100V.

B-16 melanoma cells, at 3 X 10^5 cells/ml, were incubated for 72 h with 1 µg/ml M-DNA (Figure 6, lane 1) and with 100 (lane 2), 10 (lane 3) and 1 µg/ml MCC (lane 4). M-DNA and MCC treated B-16 melanoma cells showed significant DNA fragmentation, whereas untreated B-16 melanoma cells (Figure 6, lane 5) showed no DNA fragmentation. A 123-bp DNA ladder (Gibco Life Science) was used to determine the molecular weight of the nucleosome-sized DNA fragments (Figure 6, lane L). These data show that M-DNA and MCC induce apoptosis in B-16 melanoma cells.

We claim:

1. A composition, comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the M-DNA potentiates the antineoplastic effect of the chemotherapeutic agent on cancer cells.

2. A composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the MCC potentiates the antineoplastic effect of the chemotherapeutic agent on cancer cells.

3. The composition of claims 1 or 2, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.

4. The composition of claims 1, 2 or 3 wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.

5. The composition of claim 4, wherein the cancer is melanoma.

6. The composition of claims 1, 2, 3, 4 or 5 wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

7. A method, comprising administering to an animal having cancer a composition comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the amount of the M-DNA administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.

8. A method, comprising administering to an animal having cancer a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier, wherein the amount of the MCC administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.

9. The method of claims 7 or 8, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.

10. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.

11. A method, wherein a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.

12. The method of claims 10 or 11, wherein the cell cycle arrest is induced at phase SL+GM2 of the cell cycle.

13. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.

14. A method, wherein a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.

15. A method according to claim 10, 11, 12, 13 or 14, wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.

16. A method according to claim 15, wherein the cancer is melanoma.

17. A method according to claim 10, 11, 12, 13 or 14, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

18. A method, wherein a composition comprising M-DNA and a pharmaceutically acceptable carrier is administered to an animal having melanoma in an amount effective to treat the melanoma in the animal.

19. The method of claim 18, wherein the M-DNA induces apoptosis in melanoma cells of the melanoma.

20. The method of claim 18, wherein the M-DNA inhibits proliferation of melanoma cells in the melanoma.

21. A method, wherein a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier is administered to an animal having melanoma in an amount effective to treat the melanoma in the animal.

22. The method of claim 21, wherein the MCC induces apoptosis in melanoma cells of the melanoma.

23. The method of claim 21, wherein the MCC inhibits proliferation in melanoma cells of the melanoma.

24. A method according to claim 18 or 22, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

25. A use of a composition comprising M-DNA, a chemotherapeutic agent and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.

26. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC), a chemotherapeutic agent and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer wherein the amount of the MCC administered to the animal is effective to potentiate the antineoplastic effect of the chemotherapeutic agent on cancer cells in the animal having the cancer.

27. The use of claim 25 or 26, wherein the antineoplastic effect is inhibition of proliferation of the cancer cells.

28. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.

29. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce cell cycle arrest in cancer cells in the animal having the cancer.

30. A use according to claim 28 or 29, wherein the cell cycle arrest is induced at phase SL+GM2 of the cell cycle.

31. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.

32. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having cancer in an amount effective to induce synchronization of cancer cells in the animal having the cancer.

33. A use according to claim 28, 29, 30, 31 or 32, wherein the cancer is selected from the group consisting of leukemia, lymphoma and melanoma.

34. A use according to claim 33, wherein the cancer is melanoma.

35. A use according to claim 28, 29, 30, 31 or 32, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.

36. A use of a composition comprising M-DNA and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having melanoma in an amount effective to treat the melanoma in the animal.

37. A use according to claim 36, wherein the M-DNA induces apoptosis in melanoma cells of the melanoma.

38. A use according to claim 36, wherein the M-DNA inhibits proliferation of melanoma cells in the melanoma.

39. A use of a composition comprising M-DNA preserved and complexed on *M. phlei* cell wall (MCC) and a pharmaceutically acceptable carrier in the manufacture of a medicament for administration to an animal having melanoma in an amount effective to treat the melanoma in the animal.

40. A use according to claim 39, wherein the MCC induces apoptosis in melanoma cells of the melanoma.

41. A use according to claim 39, wherein the MCC inhibits proliferation in melanoma cells of the melanoma.

42. A use according to claim 36 or 40, wherein the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous carrier and a non-aqueous carrier.